Enzyme Immunoassay Kit for Clopidol

DEIABL-QB44

1. Background
Clopidol is an organic compound that is used as in veterinary medicine as a coccidiostat. It is prepared industrially by a multistep process from dehydroacetic acid.
This kit is a new product based on ELISA technology, which is fast, easy, accurate and sensitive compared with common instrumental analysis, so it can considerably minimize operation error and work intensity.

2. Test Principle
This ELISA kit is designed to detect clopidol based on the principle of “indirect-competitive” enzyme immunoassay. The microtiter wells are coated with coupling antigen. Clopidol in the sample competes with the coating antigen for binding to the limited number of antibody added. After the addition of a ready-to-use TMB substrate the signal is measured in an ELISA reader. The absorption is inversely proportional to the clopidol concentration in the sample.

3. Applications
This kit can be used in quantitative and qualitative analysis of clopidol residue in animal tissue (pork, pork liver, chicken, chicken liver), egg and milk.

4. Cross-reactions
Clopidol..................................................100%

5. Materials required but not provided

5.1 Equipment
----Microtiter plate spectrophotometer (450nm/630nm)
----Rotary evaporator
----Homogenizer
----Vortex mixer
----Centrifuge
----Analytical balance (inductance: 0.01g
----Graduated pipette: 10ml
----Rubber pipette bulb
----Glass tube: 10ml
----Volumetric flask: 100ml, 500ml

5.2 Reagents
----Polyethylene Centrifuge tube: 2ml, 50ml
----Micropipettes: 20μl-200μl, 100μl-1000μl
250μl-multipipette

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions (5 bottles×1ml/bottle)
  0ppb, 0.5ppb, 1.5ppb, 4.5ppb, 18ppb
- Spiking standard solution: (1ml/bottle) 1ppm
- Antibody Solution 7ml…………………………green cap
- Enzyme conjugate 12ml…………………red cap
- Substrate Solution A 7ml…………………white cap
- Substrate Solution B 7ml…………………red cap
- Stop solution 7ml…………………………yellow cap
- 20×concentrated wash solution 40ml
  …………………………………….transparent cap
- 2×concentrated extraction solution 50ml
  …………………………………….blue cap

7. Solutions

Solution 1: 0.1M HCl
Dilute 4.15ml of concentrated HCl with deionized water, dilute to 500ml.

Solution 2: 10% NaCl
Dilute 10.0g of sodium chloride with deionized water and dilute to 100ml.

Solution 3: 2% TDA
Dilute 2.0g of trichloroacetic acid with deionized water and dilute to 100ml.

Solution 4: Extraction solution
Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of 1:1, which will be used for sample extraction. This solution can be conserved for a month at 4°C.
8. Sample Preparations

8.1 Notice and precautions before operation
(a) Please use one-off tips in the process of experiment, and change the tips when absorbing different reagent.
(b) Make sure that all experimental instruments are clean.
(c) Prepared tissue sample should be tested within 2h.
(d) Test liver sample should be freshly collected.

8.2 Tissue (pork, pork liver, chicken, chicken liver), egg
--- Homogenize the test sample;
--- Take 1.0±0.05g of homogenate into a 50ml polystyrene centrifuge tube;
--- Add 1ml of 0.1M HCl (solution 1), shake for 2min to dissolve thoroughly, then add 7ml of acetonitrile, shake for 3min to dissolve thoroughly;
--- Centrifuge for separation: 5min / ambient temperature / 3000g;
--- Take 1ml supernate (Do not take the impurity) into a 10ml clean glass tube, dry with 50-60℃ water bath under nitrogen gas flow;
--- Add 1ml of n-hexane, vortex for 30s, then add 1ml of extraction solution (Solution 4), vortex for 1min to mix thoroughly;
--- Centrifuge for separation: 5min / ambient temperature / 3000g;
--- Remove the upper layer of organic phase, take 50µl of the lower layer of aqueous phase for assay.

Dilution factor: 8

8.3 Raw milk
--- Take 1ml of milk sample into a 2ml polystyrene tube;
--- Add 1ml of 2% TDA (Solution 3), vortex for 1min;
--- Centrifuge for separation: 3min / ambient temperature / 7200g;
--- Take 200µl of supernate, add 800µl of extraction solution (Solution 4), vortex for 30s;
--- Take 50µl of the prepared solution for assay.

Dilution factor: 10

8.4 UHT milk
--- Take 1ml of milk sample into a 2ml polystyrene tube;
--- Add 200µl of NaCl (Solution 2) and 800µl of 2% TDA (Solution 3), vortex for 1min;
--- Centrifuge for separation: 3min / ambient temperature / 7200g;
--- Take 200µl of supernate, add 800µl of extraction solution (Solution 4), vortex for 30s;
--- Take 50µl of the prepared solution for assay.

Dilution factor: 10

9. Assay process

9.1 Notice before assay
9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).
9.1.2 Return all the rest reagents to 2-8℃ immediately after used.
9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps
9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, shake gently before use.
9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.
9.2.3 The diluted wash solution should be brought to room temperature before use.
9.2.4 Number: Numbered every microwell positions and all standards and samples should be run in duplicate. Record the standards and samples positions.
9.2.5 Add standard / sample and antibody: Add 50µl of standard solution (kit component) or prepared sample to corresponding wells. Add 50µl of antibody solution (kit component), mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover (or in dark place).
9.2.6 Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of diluted wash solution (solution 5) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
9.2.7 **Add enzyme conjugate:** Add 100µl of enzyme conjugate*(kit component)* to each well, mix gently by shaking the plate manually and incubate for 30min at 25°C with cover. Take out and **Wash the plate again following 9.2.6.**

9.2.8 **Coloration:** Add 50µl of solution A*(kit component)* and 50µl of solution B*(kit component)* to each well. Mix gently by rocking the plate manually and incubate for 15min at 25°C with cover.

9.2.9 **Measure:** Add 50µl of stop solution*(kit component)* to each well. Mix gently by rocking the plate manually and measure the absorbance at 450nm *(It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.)*

10. **Results**

10.1 **Percentage absorbance**
The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

\[
\text{Absorbance (\%)} = \frac{B}{B_0} * 100
\]

B —— absorbance standard (or sample)
B₀ —— absorbance zero standard

10.2 **Standard Curve**
---To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the clopidol standards solution (ppb) as x-axis.
---The clopidol concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

**Notice:** *Analysis software can be provided on request.*

11. **Sensitivity, accuracy and precision**

**Test Sensitivity:** 0.5 ppb
**Detection limit**
Animal tissue, egg……………………………………..4ppb
Milk ……………………………………………………..5ppb

**Accuracy**
Animal tissue, egg, milk……………………………………100±20%

**Precision:**
CV of the ELISA kit is less than 10%.

12. **Notice**

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful reproducibility and operate the next step immediately after tap the microwells holder.

12.3 Shake each reagent gently before use.

12.4 Keep your skin away from the stop solution for it is the 0.5M H₂SO₄ solution.

12.5 Don’t use the kits out of date. Don’t exchange the reagents of different batches; otherwise it will drop the sensitivity.

12.6 Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates, Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Substrate solution should be abandoned if it turns colors. The reagents may be deteriorated if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A₄₅₀nm<0.5).

12.8 The coloration reaction needs 15min after the addition of solution A and solution B. You can prolong the incubation time to 20min or more if the color is too light to be determined. Never exceed 30min. On the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. **Storage**

**Storage condition:** 2-8°C.
**Storage period:** 12 months.